Group: 12

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(54) **7-aminocephalosporanic Acid Derivatives and Process for Preparing Them**

The invention refers to 7-aminocephalosporanic acid derivatives useful as antibiotics and to a process for preparing them.

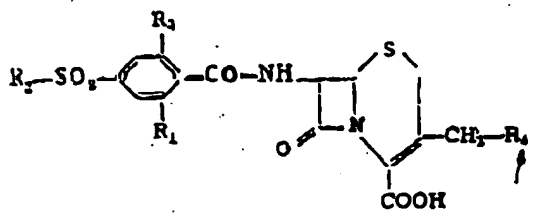
Numerous derivatives of cephalosporine with substituents in positions 3 and 7 are known, such as 7- or thio-aminocephalosporin containing in position 3 an acetoxy or 2-methyl-1,3,4-thiadiazole-5-ilthio group substituted in the p-sulfonyl group by a pyrolidine, piperidine, morpholine, respectively, N-methylpiperazine, iethylamine, or diisopropylamine radical (Romanian patents 81632 and 81633). These are obtained by acylation of the respective 7-aminocephalosporine with the chloride of the sulfonylphenyl oxy- or corresponding thio-acetic acids substituted in a short-chain halogenated aliphatic hydrocarbon medium at an initial temperature of 3-5°C and then at 20-25°C in the presence of a base.

Also similarly obtained are 7-sulfonyl phenoxy-acetamido desacetoxy cephalosporins substituted in the sulfonyl group by diethylamine, diisopropylamine, pyrolidine, morpholine, or piperidine (Romanian patent 85919).

The object of the invention is to extend the range of cephalosporins with new compounds.

The problem solved by the invention consists in establishing new associations of raw materials under certain reaction conditions.

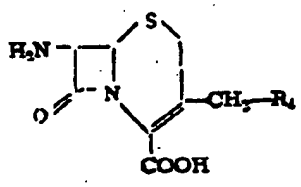
According to the invention, the chemical structure of the derivatives corresponds to the general formula I:



(I)

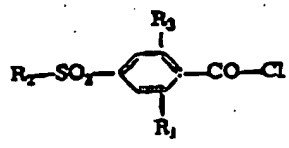
where R_1 represents a methyl or methoxy group, or a chloride atom when R_3 is a hydrogen atom or R_1 and R_3 each represent a methoxy group. R_2 represents a diethylamine, dipropylamine, diisopropylamine, dibutylamine, morpholine, piperidine, or pyrolidine group, R_4 represents a hydrogen atom, an acetoxy or 2-methyl-5-il [ilthio]-mercapto-1,3,4-thiadiazole group and are solids with IR absorption maxima (KBr) between 1750-1770 cm^{-1} corresponding to the -CO-lactamic group, between 1660-1678 cm^{-1} corresponding to the -CO-amidic group, between 1158-1180 cm^{-1} corresponding to the -CO-carbonylic group, and with an Rf value of 0.34-0.56.

Their preparation process consists of treating a 7-aminocephalosporin whose general formula is II:



(II)

and whose R_4 has the connotations described above, with an acylation agent whose general formula is III:



(III)

where R_1, R_2, and R_3 have the connotations described above, the reaction occurring in a short-chain halogenated aliphatic hydrocarbon medium, preferably anhydrous methylene chloride as inert solvent, at an initial temperature of 3-5°C and then at 15-20°C in the presence of a hydrochloric acid acceptor selected between triethylamine and dimethylaniline, after which the product is isolated and purified by extraction in acid *p*H, by means of well known processes.

Following are three examples of the implementation of the invention.

Example 1. *7-(2-chlor-4-morpholinosulfonyl-benzamido)-cephalosporanic acid.*

1 g of 7-aminocephalosporanic acid (95% pure) is suspended in 15 ml of methylene chloride and 1 ml of triethylamine is added while under agitation. Agitation is continued until dissolution, after which the solution obtained is cooled to 3-5°C and a solution created by dissolving one gram of 2-chlor-5-morpholino-sulfonyl benzoic acid chloride in 15 ml of methylene chloride is slowly added.

After the addition of the acid chloride, the acylation reaction is carried out for 60 min. at a temperature of 15-20°C. The solution is then cooled to 0-5°C and the *p*H is brought to a value of 2-2.5 with a 5% HCl solution, after which the phases are separated.

The organic phase is washed with 2 ml of cold water, after which the organic phase is treated with 1 g of activated charcoal and 10 g of anhydrous sodium sulfate, and then filtered.

To the obtained filtrate are added 2.3 ml of 26.4% isopropanol solution of sodium 2-ethylhexanoate, after which the mixture is concentrated twice in vacuum.

3-4 volumes of petroleum ether are added to the obtained concentrate while under agitation when the white-cream colored cephalosporin sodium salt precipitates. The product is filtered and washed with 10 ml of petroleum ether, and dried in vacuum.

1.5 g of product with a chromatographically measured purity of 90% are obtained. The yield is 66.6%.

Example 2. *7-(2-methyl-4-piperidine sulfonyl-benzamido)-3-(2-methyl-1,3,4-thiadiazole-5-il* [translators' note:typo?]*-thiomethyl)-cephalosporanic acid.*

1.1 g of 3-(2-methyl)-1,3,4-thiadiazole-5-il [translators' note:typo?]-thiomethyl)-cephalosporanic acid is suspended in 15 ml of anhydrous dichloromethane to which are then added 0.5 ml of hexamethyldisilazane and 0.25 ml of trimethylchlorosilane and lastly 0.5 ml of dimethylaniline, while cooled to 3-5°C.

Agitation is maintained until complete dissolution at room temperature. A solution obtained by dissolving one gram of 4-piperidine sulfonyl-2-methylbenzoic acid chloride in 15 ml of dichloromethane is slowly added into the reaction mixture thus obtained.

The acylation reaction is carried out for 2 hrs. at a temperature of 15-20°C, after which the mixture is acidulated to a *p*H of 1.5-2 with a 5% hydrochloric acid solution. After decanting, the phases are separated and the aqueous phase is washed with an additional 10 ml of methylene chloride.

The joint organic phases are washed with a volume of cold water, after which they are discolored with 1 g of activated charcoal and anhydrated with 10 g of anhydrous sodium sulfate.

2.3 ml of 26.4% sodium 2-ethylhexanoate in isopropanol are added, the mixture is concentrated twice in vacuum, after which 2 volumes of petroleum ether are added while under agitation when the cephalosporin sodium salt crystallizes in the form of white crystals. After filtering, the product is washed with 10 ml of petroleum ether and finally dried in vacuum. 1 g of product with a purity of 90% is obtained. The yield of 7-ACA (7-aminocephalosporanic acid) is 44.6%.

Example 3. *7-(2-chlor-4-diisopropylamine sulfonyl-benzamido)-desacetoxy cephalosporanic acid.*

0.5 g of 7-aminodesacetoxy cephalosporanic acid is suspended in 10 ml of anhydrous dichloromethane and is treated with 0.5 ml of hexamethyl disilazane after which the obtained mixture is refluxed for 90 minutes until complete dissolution.

The obtained solution is cooled to 5-8°C, 0.3 ml of dimethylaniline is added, after which a solution composed of 1 g of 2-chlor-5-diisopropylamine sulfonyl benzoic acid chloride in 15 ml of dichloromethane is introduced slowly, drop by drop, while under agitation.

After addition of the acid chloride, agitation is continued at room temperature for 90 minutes, and then the mixture obtained is cooled to 3-5°C. 10 ml of cold water are then added, agitation is maintained for 10 minutes, and the *p*H is brought to a value of 2 with a 5% HCl solution. The phases are then separated.

The short-chain organic phase is washed with 10 ml of cold water, decanted, and the phases are separated.

The organic phase is treated with 10 g of anhydrous sodium sulfate and 1 g of activated charcoal, and filtered.

The solution thus obtained is treated with 1.4 ml of an isopropanol solution of 26.4% sodium 2-ethylhexanoate, the obtained mixture is concentrated twice in vacuum, after which it is treated while under agitation with 2.5 volumes of petroleum ether.

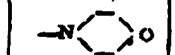
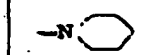
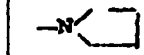
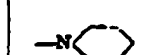
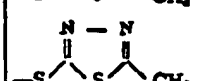
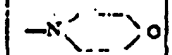
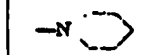
The crystals obtained are filtered in vacuum and washed on the filter with 25 ml of petroleum ether. They are dried in vacuum

1 g of product with a purity of 90% is obtained. The yield of 7-ACA (7-aminocephalosporanic acid) is 75.7%.

The cephalosporins of the formula I structure were characterized with IR spectra (KBr pellet) that exhibited characteristic maxima. Thin-layer chromatography (silicagel F-254, 0.3 ml of NaCl mobile phase solution) was used to determine the Rf value.

Table 1 presents the physicochemical data of the cephalosporins that are the object of the present invention.

Table 1

Nr. crt.	R_1	R_2	R_3	R_4	IR absorption maxima				Rf	yield %
					-CO-lactamic	-CO-imidic	-CO-carboxilic	$-SO_2-N$		
1.	2-Cl		H	$-OCOCH_3$	1770	1670	1600	1170	0,48	66,7
2.	2-OCH_3	$-N(C_3H_7)_2$	6-OCH_3	$-OCOCH_3$	1770	1680	1590	1160	0,40	52,0
3.	2-CH_3		H	$-OCOCH_3$	1760	1670	1600	1170	0,42	63,3
4.	3-Cl		H	$-OCOCH_3$	1770	1680	1610	1160	0.50	57,1
5.	2-CH_3		H		1760	1660	1600	1160	0,35	44,6
6.	2-OCH_3		6-OCH_3		1760	1678	1610	1160	0,56	65
7.	2-Cl	$-N(C_6H_5)_2$	H		1760	1680	1610	1180	0,50	89,7
8.	2-Cl		H		1758	1670	1600	1160	0,36	85
9.	2-Cl	$-N(iC_3H_7)_2$	H	H	1758	1670	1600	1160	0,36	75,7
10.	2-CH_3		H	H	1760	1660	1600	1170	0,4	73
11.	2-OCH_3		6-OCH_3	H	1758	1670	1570	1160	0,34	66,1
12.	2-Cl	$-N(C_2H_5)_2$	H	H	1750	1660	1600	1158	0,42	68,2

The present invention expands the range of 7-aminocephalosporanic acid derivatives by presenting compounds with antibiotic action toward *gram*-positive and *gram*-negative bacteria.

Cephalosporins of the general formula I are obtained through the acylation of a 7-aminocephalosporin of the general formula II with an acylation agent corresponding to the general formula III, in an inert anhydrous medium composed of a short-chain halogenated aliphatic hydrocarbon such as dichloromethane or chloroform, preferably dichloromethane, at a temperature of 0-10°C, preferably 3-5°C, in the presence of a hydrochloric acid acceptor such as dimethylaniline or triethylamine, preferably triethylamine.

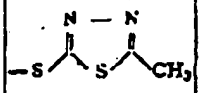
Compound II is solubilized in the reaction medium as triethylammonium salt or silyl ester, as a function of the nature of group R_4, by treating with triethylamine or silanes, such as for instance trimethylchlorosilane or hexamethyldisilazane.

The cephalosporins resulting from the acylation reaction are isolated under acid *p*H in an organic solvent composed of the chlorinated aliphatic hydrocarbon in which the acylation reaction occurred, preferably in dichloromethane, followed by a transformation in a pharmaceutically acceptable sodium salt, with the aid of sodium 2-ethylhexanoate or sodium acetate, preferably sodium 2-ethylhexanoate in isopropanol.

Since the sodium salts of formula I cephalosporins are soluble in methylene chloride, chloroform, or acetone, their isolation from the reaction medium is performed by the addition of a nonsolvent such as butyl alcohol, ethyl ether, or petroleum derivatives of the benzene or petroleum ether type, preferably petroleum ether.

The antimicrobial action of some of the cephalosporins of the general formula I is presented in Table 2.

Table 2. *Antimicrobial activity of some cephalosporins (CMI in µg/ml)*

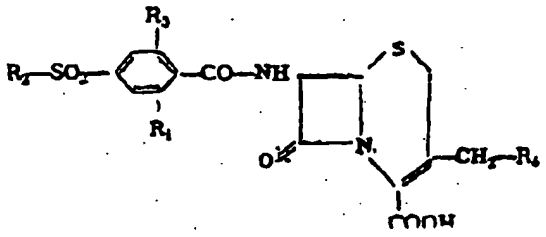
Nr. crt.	R_1	R_2	R_3	R_4	C.M.I. µ g/ml			
					A	B	C	D
1.	2-Cl		H	$-OCOCH_3$	>50	10	>50	>50
2.	2-Cl	$-N(C_2H_5)_2$	H	$-OCOCH_3$	10	10	>50	>50
3.	2-OCH_3		6-OCH_3		>50	>50	>50	>50
4.	2-OCH_3		6-OCH_3	H	>50	>50	>50	>50
5.	2-CH_3		H	H	>50	>50	>50	>50
6.	2-Cl	$-N(C_2H_5)_2$	H	H	>50	>50	>50	>50

The values in the table represent the minimum inhibitory concentrations (C.M.I.) against the indicated microorganisms.

The values were obtained by the method of serial dilutions on solid medium with respect to 4 test germs: *Bacilus subtilis A, Staphylococcus aureus B, Escherichia coli C,* and *Klebsiella pneumoniae D.*

Claims

1. Derivatives of 7-aminocephalosporanic acid, characterized in that they have a chemical structure corresponding to the general formula I:

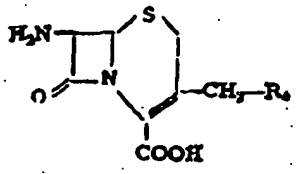


(I)

where R_1 represents a methyl or methoxy group, or a chloride atom when R_3 is a hydrogen atom or R_1 and R_3 each represent a methoxy group. R_2 represents a diethylamine, dipropylamine, diisopropylamine, dibutylamine, morpholine, piperidine, or pyrolidine group, R_4 represents a

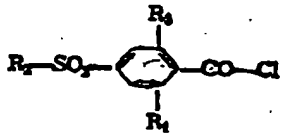
hydrogen atom, an acetoxy or 2-methyl-5-il [translators' note: typo for ilthio?]-mercapto-1,3,4-thiadiazole group and are solids with IR absorption maxima (KBr) between 1750-1770 cm^{-1} corresponding to the -CO-lactamic group, between 1660-1678 cm^{-1} corresponding to the -CO-amidic group, between 1158-1180 cm^{-1} corresponding to the -CO-carbonylic group, and with an Rf value of 0.34-0.56.

2. Preparation process for the derivatives according to claim 1, characterized in that a 7-aminocephalosporin whose general formula is II:



(II)

where R_4 has the connotations described above, is treated with an acylation agent of the general formula III:



(III)

where R_1, R_2, and R_3 have the connotations described above, the reaction occurring in a short-chain halogenated aliphatic hydrocarbon medium, preferably anhydrous methylene chloride as inert solvent, at an initial temperature of 3-5°C and then at a temperature of 15-20°C in the presence of a hydrochloric acid acceptor selected between triethylamine and dimethylaniline, after which the product is isolated and purified by extraction in acid *p*H, by means of well known processes.

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